

DEVELOPMENT OF ALGINATE BASED ANTIMICROBIAL HEMOSTATIC DRESSINGS

ANKITA SHARMA



**DEPARTMENT OF TEXTILE AND FIBRE ENGINEERING
INDIAN INSTITUTE OF TECHNOLOGY DELHI**

APRIL 2024

DEVELOPMENT OF ALGINATE BASED ANTIMICROBIAL HEMOSTATIC DRESSINGS

by

ANKITA SHARMA

Department of Textile and Fibre Engineering

Submitted

in fulfilment of the requirements of the degree of

Doctor of Philosophy

to the



INDIAN INSTITUTE OF TECHNOLOGY DELHI

April 2024

DEDICATED TO MY PARENTS

CERTIFICATE

This is to certify that the thesis entitled '**Development of Alginate based Antimicrobial Hemostatic Dressings**' submitted by **Ms. Ankita Sharma** to the **Indian Institute of Technology Delhi** for the award of degree **Doctor of Philosophy**, is a record of bonafide research work carried out by her. She has worked under our guidance and supervision and fulfilled the requirements for the submission of this thesis which has attained the standard required for a Ph.D. degree of this institute. The results contained in this thesis are original and have not been submitted, in part or in full, to any other university or institute for the award of any degree or diploma.



Prof. Bhuvanesh Gupta

Department of Textile and Fibre Engineering

Indian Institute of Technology Delhi

New Delhi, India



Prof. Samrat Mukhopadhyay

Department of Textile and Fibre Engineering

Indian Institute of Technology Delhi

New Delhi, India



Dr. Amlan Gupta

Department of Pathology

Sikkim Manipal Institute of Medical Sciences

Gangtok, Sikkim, India

ACKNOWLEDGEMENTS

I am immensely grateful to God for the opportunities and blessings bestowed upon me and providing me the strength and perseverance throughout this research journey. I would like to express my sincere gratitude and appreciation to the following individuals who have been instrumental in the completion of my thesis. Without their support it would have been impossible for me to accomplish this thesis. That is why I would like to dedicate this section to recognize their support.

First and foremost, I would like to extend my heartfelt thanks and indebtedness to my venerated supervisors, **Prof. Bhuvanesh Gupta** (Department of Textile and Fiber Engineering, IIT Delhi, New Delhi, India), **Prof. Samrat Mukhopadhyay** (Department of Textile and Fiber Engineering, IIT Delhi, New Delhi, India), and **Dr. Amlan Gupta** (Department of Pathology, Sikkim Manipal Institute of Medical Sciences, Gangtok, Sikkim, India), for their invaluable guidance, unwavering support, and insightful feedback. Their expertise, patience, vast store of knowledge, and encouragement have been indispensable in shaping this research project and enhancing my academic growth. Their dedication to my success has been inspiring and I am really honoured to have such remarkable supervisors. They have always been a patient bearer of my mistakes. Words cannot describe the kindness and support I received from them. I am truly fortunate to have had the privilege of working under their mentorships.

I extend my sincere thanks to SRC members: Prof. Harpal Singh, Prof. Manjeet Jassal, and Prof. Rajiv Srivastava for their invaluable suggestions, critical reviews, and untiring help in designing the experimental strategies.

I would like to acknowledge IIT Delhi for providing me financial assistance throughout my Ph.D. tenure and “Research Scholar Travel Award (RSTA)” for attending the international conference. I also want to express my gratitude to Industrial Research & Development Unit,

IIT Delhi, for providing me the “Research Excellence Travel Award (RETA)” travel grant for attending international conference.

I would like to express my sincere regards to the Head of the Department (HOD), Prof. R. Alagirusamy and all the respected faculty members of the Department of Textile and Fibre Engineering for their cooperation of knowledge sharing during teaching assistantship and in smooth completion of my academic work. I would also like to acknowledge the facilities provided by the department and the supportive cooperation received from the departmental staff Mr. Amarjeet, Mr. V. K. Kala, Mr. R. Khattar, Mr. Shiv Upadhyay, Mr. Rohit Kumar, Mr. Sandeep Kumar, Dr. Virendra Sharma, and Dr. Vikas Khatkar.

I am also thankful to all CRF staff members especially to Mr. Kuldeep Sharma, Mr. Shiv Solanki, Mr. Dinesh Kumar, Mr. Mahesh Soni, Ms. Aastha Sharma, and Ms. Kumud Arora for their support during the instrumentation and characterizations of samples.

I have been very fortunate to be a part of great research team. I extend my deep gratitude to my lab colleagues (Dr. Chetna Verma, Ms. Pratibha Singh, Ms. Vandana Kumari, Ms. Manali Somani, Ms. Vipula Sethi, Ms. Rohini Verma) who have been a great support professionally and personally throughout this challenging endeavour and for maintaining the work friendly environment of the lab. Time to time celebrations for the research outcomes motivated me and allowed me to carry out this work with enthusiasm. The memories we created here are deeply rooted in my heart. I cannot express my gratitude in words for Dr. Chetna Verma for all the support and guidance during the ups and downs of this work and through the journey. Her continuous support and encouragement was like the strong pillar all the time. I would like to add special words of thanks to Ms. Pratibha Singh for sharing all the laughter and tears together and for making my life here a memorable one as we had a wonderful time together. She was always there to provide me a shoulder to lean on during times of thick and thin. Her support during the experimentations was appreciable.

I would like to express my gratitude to Ms. Prashansa Dalal for helping me in the career guidance. I deeply acknowledge the support received from Dr. Sonia Mann (CSIR- Institute of Genomics and Integrative Biology, Delhi, India) during the selection of herbal plants and their extract preparation. She has always been there to provide me unspoken support in my journey. I am also thankful to Dr. Rohit Sarda (Sikkim Manipal Institute of Medical Sciences, Gangtok, Sikkim, India) for helping in conducting the animal studies at SMIMS, Gangtok, Sikkim, India. I would like to thank Mr. Anees, Mr. Sumit Kapoor, and Ms. Parna Nandi for their endless help at every juncture of need. I would like to extend my heartfelt thanks to Dr. Shachi Mathur for helping me in the stress management during this journey.

I am also indebted to my loving parents, Mr. S. K. Sharma and Mrs. Nirmal Sharma for making me capable of reaching this height of education. My sister, Ms. Manu Sharma, Brother-in-law, Mr. Ankur Sharma, and two bundle of joy Aaryash and Aaryana for their unconditional love, belief in my abilities, and continuous encouragement. I am deeply grateful to my family for their patience, understanding, and motivation that have carried me through the ups and downs of this thesis journey.

Additionally, a stock of loving appreciation is reserved for my friends (Simran, Monika, Priyal, Gunjan, Dr. Rupsi Kharb, Varsha, Rangoli, Kshama) for solace during times of stress and have made this journey more enjoyable.

To everyone mentioned above and to whom I have missed to mention their names but have been a part of this thesis journey in any way, I extend my heartfelt gratitude. Your support, encouragement, and belief in me have been invaluable, I am truly grateful for the profound impact you have had on my academic and personal growth.

Thank you.

Ankita Shrama

ABSTRACT

Uncontrolled bleeding is acknowledged as primary cause of civilian and war field deaths before hospitalization and is also known for the debilitation of wound healing process. Irrepressible bleeding is also an inevitable reason for microbial infection. These challenges necessitate the development of materials with multifunctional properties of immediate blood clotting. Thus, present study was undertaken to develop herbal incorporated alginate based antimicrobial hemostatic cotton dressings for immediate blood clotting and antimicrobial activity.

In order to impart the infection resistant activity to hemostatic dressings, the synthesis of nano silver encapsulated carboxymethyl cellulose gels (CMC-Ag nanogels) was carried out. Silver nitrate was reduced and stabilized by the fructose and CMC, respectively. Preparation of nanogels is influenced by the reaction conditions, such as stabilizing agent concentration, reduction time, and reduction temperature. The phenomenon of agglomeration was observed for the higher concentrations of CMC due to high viscosity of reaction mixture, whereas, the low concentration was found to be insufficient for stabilizing the nanogels. In addition to this, the short reduction time showed incomplete reduction of ionic silver while, particle aggregation was observed when reduction was allowed for longer duration. However, the effect of reduction temperature leads to the formation of spherical nanogels which aggregated upon increasing the reduction temperature. The morphological analysis of CMC-Ag nanogels was investigated through HR-TEM analysis which indicated the core shell structure formation in the size range of 5-10 nm. Encapsulation of nano silver inside CMC matrix provided the functional groups to CMC-Ag nanogels for adhering on the cotton fabric. Optimized CMC-Ag nanogels were immobilized on the cotton fabric by varying the concentration of silver nitrate. These fabrics were investigated for their antimicrobial activity against *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) bacterial strains by zone of inhibition, anti-adhesion analysis, time kill assay,

and colony count method where >99% bacterial colony reduction for CMC-Ag300 fabric (which was further designated as NG during the fabrication of hemostatic dressings).

To introduce the hemostatic activity, sodium alginate (SA) based blends were prepared with different content of glycerol (Gly). Rheological studies were conducted to investigate the flow behavior of blend system which revealed decrease in the viscosity upon increasing the Gly concentration. Contact angle analysis showed an increase in the hydrophilicity with the increase in the Gly content. Flexibility of films increased while the tensile strength decreased with the increase in the Gly content. These observations were attributed to the presence of Gly which enhanced polymer chain mobility by disturbing the intermolecular hydrogen bonding in SA. SA:Gly blends were coated on the cotton fabric where Gly content offered good flexibility to the fabric, similar to the pristine cotton gauze. The optimized blend of SA:Gly was designated as the SG for hemostatic dressing preparation.

To fabricate the antimicrobial hemostatic dressings, varying concentrations of three herbal extracts (Tannic Acid (TA), *Choerospondias axillaris* (CA), and *Mesua ferrea* (MF)) were added to the SG blend followed by their coating on the NG fabric by dip coat method. DPPH analysis showed an excellent antioxidant activity of developed dressings in a dose dependent manner. Presence of herbal extracts helped in clotting the blood faster than the control sample as measured by *in-vitro* blood clotting time analysis. FESEM analysis revealed the presence of stable clot with fibrin mesh on optimized dressings. The infection resistant behavior of the dressings was studied by the zone of inhibition, anti-adhesion analysis, and colony count method which exhibited more than 97% of viable colony reduction against *S. aureus* and *E. coli*. Additionally, dead bacterial cells were detected on the surface of dressing due the excellent antiadhesion activity. Biocompatibility of the dressings was delineated with MTT

assay on NIH3T3 cells which implicated more than 85% of cell viability suggesting their cytocompatible nature.

A comparative study of hemostatic activity of all optimized dressings with TA, CA, and MF (NGSG-MF3, NGSG-TA3, and NGSG-CA3) was carried out by *in-vivo* tail amputation test. Swiss albino mice were used for the analysis which indicated faster blood clotting in NGSG-MF3, NGSG-TA3, and NGSG-CA3 groups as compared to the other groups. Blood clotting efficiency was measured by the hemostatic time (s) and blood loss (g) which revealed that NGSG-CA3 dressing has the fastest clotting time of 66.37 ± 17.32 s and minimum blood loss of 0.135 ± 0.03 g. This investigation led to the development of a hemostatic dressing by combining SA, gly and CA as the hemostatic compound.

सार

अनियंत्रित रक्त स्राव को युद्ध: क्षेत्र में होने वाली मौतों का प्राथमिक कारण माना जाता है। यह माइक्रोबियल संक्रमण को आमंत्रित करता है जिसकी वजह से घाव भरने की प्रक्रिया की गति धीमी हो जाती है। इन चुनौतियों को ध्यान में रखते हुए ऐसी ड्रेसिंग्स बनानी की आवश्यकता है जिनके पास तत्काल रक्त के थक्के जमाने, शारीरिक कोशिकाओं के अनुकूल, और संक्रमण से निपटने की क्षमता हो। हेमोस्टैटिक ड्रेसिंग्स बनाने के लिए कॉटन गोर्जेस को अनुकूल माना जाता है क्योंकि इसके पास द्रव्य को सोखने की अच्छी क्षमता होती है जिससे यह रक्त के कंपोनेंट्स को एकत्र करने तथा घाव को बाहरी चोटों से बचाने की शक्ति रखता है। इस प्रकार, इस काम में हमने हर्बल एक्सट्रेक्ट युक्त अल्लिनेट की रोगाणुरोधी रक्त प्रवाह को रोकने वाली ड्रेसिंग्स बनायीं हैं।

अपने द्वारा बनायीं जाने वाली हेमोस्टैटिक ड्रेसिंग्स को रोगाणुरोधी क्षमता देने के लिए हमने कार्बोक्सीमिथइल सेलुलोस की परत चढ़े नैनो सिल्वर जेल बनाये। इन नैनो जेल्स को बनाने के लिए सिल्वर नाइट्रेट को फ्रुक्टोज और सीएमसी की उपस्थिति में नैनो साइज में बदला गया जिसमें फ्रुक्टोज ने नैनो साइज में बदला तथा सीएमसी ने इन्हे स्थिर करने का काम किया। नैनो जेल बनाने की प्रक्रिया में रिएक्शन की स्थिति (जैसे सीएमसी की कॉन्सन्ट्रेशन, रिएक्शन का समय, रिएक्शन की तापमान) की बहुत बड़ी भूमिका होती है। स्थिर नैनोजेल बनाने के लिए स्थिरीकरण एजेंट की एक इष्टतम सांद्रता की आवश्यकता होती है। सीएमसी की निचली और उच्च सांद्रता के लिए संचयन की घटना देखी गई, जहां कम सांद्रता नैनोजेल को स्थिर करने के लिए अपर्याप्त पाई गई और उच्च सांद्रता ने प्रतिक्रिया मिश्रण की चिपचिपाहट को बढ़ा दिया। इसके अलावा, रिएक्शन समय विश्लेषण से संकेत मिलता है कि शुरुआती समय में आयनिक सिल्वर की अधूरा रिडक्शन देखा गया, जबकि लंबी अवधि के लिए रिडक्शन की अनुमति देने पर जेल का एकत्रीकरण देखा गया। हालाँकि, रिएक्शन की तापमान के विश्लेषण से पता चला कि तापमान में कमी के प्रभाव से गोलाकार आकार के नैनोजेल का निर्माण हुआ जो तापमान बढ़ने पर बड़े आकार में एकत्र हो गए। सीएमसी-एजी नैनोजेल के रूपात्मक विश्लेषण की जांच एचआर-टीईएम विश्लेषण के माध्यम से की गई, जिसने 5-10 एनएम की आकार सीमा में कोर शेल संरचना के गठन का संकेत दिया। कार्बोक्सीमिथइल सेलुलोस की परत नैनो सिल्वर को कॉटन गौज पे चिपकने के लिए कार्यात्मक समूह प्रदान करती है। इसके अलावा, सिल्वर नाइट्रेट की भिन्न-भिन्न सांद्रता को अनुकूलित सीएमसी-एजी नैनोजेल में डालकर कॉटन गौज पे चिपकाया गया। इन गौज की जांच ई. कोली (ग्राम-नेगेटिव) और एस. ऑरियस (ग्राम-पॉजिटिव) बैक्टीरियल संक्रमण के खिलाफ उनकी रोगाणुरोधी गतिविधि के लिए ज़ोन ऑफ़ इन्हिबीशन, एंटी ऐडहेशन, टाइम किल, तथा कॉलोनी काउंट विधि की मदद से की गई थी। CMC-Ag300 फैब्रिक के लिए >99% बैक्टीरियल कॉलोनी में कमी का पता चला (जिसे हेमोस्टैटिक ड्रेसिंग के निर्माण के दौरान एनजी के रूप में नामित किया गया था)।

अपनी ड्रेसिंग्स में हेमोस्टैटिक गुण और लचीलापन लाने के लिए हमने सोडियम अल्लिनेट और ग्लिसरॉल के मिश्रण का अध्ययन करके अनुकूल सांद्रता का पता लगाया। मिश्रण प्रणालियों के प्रवाह व्यवहार की जांच करने के लिए रियोलॉजिकल अध्ययन आयोजित किया गया, जिसमें ग्लिसरॉल की सांद्रता बढ़ने पर चिपचिपाहट में

कमी का पता चला। संपर्क कोण विश्लेषण से ग्लिसरॉल की मात्रा में वृद्धि के साथ हाइड्रोफिलिसिटी में वृद्धि देखी गई। इसके अलावा, फिल्मों का लचीलापन बढ़ गया जबकि ग्लिसरॉल में वृद्धि के साथ फिल्मों की तन्यता ताकत कम हो गई। इन अवलोकनों को ग्लिसरॉल की उपस्थिति के लिए जिम्मेदार ठहराया गया था जो एसए के अंतर-आणविक हाइड्रोजन बांड को कमजोर करके बहुलक शृंखला की गतिशीलता को बढ़ाता था। एसए:ग्लिसरॉल के मिश्रणों को ऐड-ऑन प्रतिशत विश्लेषण के लिए कॉटन गौज़ पर लेपित किया गया, जिससे पता चला कि मिश्रणों में ग्लिसरॉल की सांद्रता बढ़ने पर (चिपचिपाहट कम होने के कारण) इसमें कमी आई। हमारे अध्ययन से पता चला कि ग्लिसरॉल की ३०% सांद्रता मूलरूप कॉटन गौज़ के समान लचीलेपन को बनाए रख रही थी और इसमें $4.03 \pm 0.28\%$ का ऐड-ऑन प्रतिशत था, इस मिश्रण को आगे के विश्लेषण के लिए अनुकूलित किया गया था। एसए:ग्लिसरॉल के इस अनुकूलित मिश्रण को हेमोस्टैटिक ड्रेसिंग विकास के लिए एसजी के रूप में नामित किया गया।

रोगाणुरोधी हेमोस्टैटिक ड्रेसिंग बनाने के लिए, तीन हर्बल अर्क (टीए, सीए और एमएफ) की अलग-अलग सांद्रता को एसजी मिश्रण में डाला गया और उसके बाद डिप कोट विधि द्वारा एनजी गौज़ पर उनकी कोटिंग की गई। विकसित ड्रेसिंग की डीपीपीएच विश्लेषण ने सांद्रता के अनुसार एंटीऑक्सीडेंट गतिविधि दिखाई। हर्बल अर्क वाली हेमोस्टैटिक ड्रेसिंग ने इन-विट्रो रक्त के थक्के जमने के समय विश्लेषण में कंट्रोल की तुलना में तेजी से रक्त का थक्का जमाने में मदद की, जबकि सबसे कम बीसीआई% के कारण, एनजीएसजी-एमएफ ३, एनजीएसजी-टीए ३ और एनजीएसजी-सीए ३ ड्रेसिंग को अनुकूलित किया गया था। एफईएसईएम विश्लेषण से अनुकूलित ड्रेसिंग पर फाइब्रिन जाल के साथ स्थिर थक्के की उपस्थिति का पता चला। इसके अलावा, अनुकूलित ड्रेसिंग की संक्रमण प्रतिरोधी गतिविधि को ज़ोन ऑफ़ इन्हिबीशन, एंटी ऐडहेशन, तथा कॉलोनी काउंट विधि द्वारा जांचा गया, जिसमें एस. ऑरियस और ई. कोली बैक्टीरियल उपभेदों के खिलाफ ९७% से अधिक मारने की क्षमता देखी गई। इसके अलावा, उत्कृष्ट एंटीएडिशन गतिविधि के कारण ड्रेसिंग सतह पर मृत जीवाणु कोशिकाएं देखी गई। ड्रेसिंग की बायोकम्पैटिबिलिटी को एनआईएच३टी३ कोशिकाओं पर एमटीटी के साथ अध्ययन किया गया था, जिसमें ८५% से अधिक सेल प्रसार शामिल था, जो उनकी साइटोकंपैटिबल प्रकृति की ओर इशारा करता है।

इन-विवो टेल एम्प्यूटेशन टेस्ट द्वारा उनकी हेमोस्टैटिक गतिविधि के आधार पर सभी अनुकूलित ड्रेसिंग (एनजीएसजी-एमएफ ३, एनजीएसजी-टीए ३ और एनजीएसजी-सीए ३) का तुलनात्मक अध्ययन किया गया। विश्लेषण के लिए स्विस अल्बिनो चूहों का उपयोग किया गया, जिसमें अन्य समूहों की तुलना में एनजीएसजी-एमएफ३, एनजीएसजी-टीए३ और एनजीएसजी-सीए३ समूहों में तेजी से रक्त का थक्का जमने का संकेत मिला। रक्त के थक्के जमने की दक्षता को हेमोस्टैटिक समय (सेकंड) और रक्त छति (ग्राम) द्वारा मापा गया था, जिससे पता चला कि एनजीएसजी-सीए ३ ड्रेसिंग में सबसे तेज़ थक्का जमने का समय 66.37 ± 17.32 सेकंड और न्यूनतम रक्त छति 0.135 ± 0.03 ग्राम है।

TABLE OF CONTENTS

CONTENTS	Page No.
Certificate	i
Acknowledgements	ii
Abstract	v
Table of Contents	x
List of Figures	xv
List of Tables	xxi
List of Abbreviations	xxii
 Chapter 1: Introduction and Literature Review	
1.1 Introduction	1
1.2 Overview of Hemostasis	3
1.3 Commercially Available Hemostats	4
1.4 Hemostatic Materials	6
1.4.1 Characteristic Features of Hemostatic Material	6
1.4.2 Types of Hemostatic Materials	7
1.4.2.1 Polymer based Hemostatic Materials	7
1.4.2.2 Biologically Derived Hemostatic Materials	11
1.4.2.3 Synthetic Drug based Hemostatic Materials	12
1.4.2.4 Inorganic Compound based Hemostatic Materials	13
1.4.2.5 Plant based Hemostatic Materials	15
1.4.3 Sodium Alginate as Hemostatic Material	17
1.4.4 Herbal Hemostatic Materials	19
1.5 Objectives of the Work	22

1.6 Format of the Thesis	22
1.7 References	24
Chapter 2: Preparation of Nano Silver Encapsulated Carboxymethyl Cellulose Gels and their Immobilization	
2.1 Introduction	33
2.2 Experimental	36
2.2.1 Materials	36
2.2.2 Synthesis of CMC-Ag Nanogels	36
2.2.2.1 Effect of CMC Concentration	36
2.2.2.2 Effect of Reduction Time	37
2.2.2.3 Effect of Reduction Temperature	37
2.2.3 Characterizations	37
2.2.3.1 Energy-Dispersive X-ray Spectroscopy (EDX) Analysis	37
2.2.3.2 UV-vis Spectroscopy Analysis	37
2.2.3.3 Transmission Electron Microscopy (TEM) Analysis	37
2.2.3.4 Field Emission Scanning Electron Microscopy (FESEM) Analysis	38
2.2.3.5 Dynamic Light Scattering (DLS) Analysis	38
2.2.4 Immobilization of CMC-Ag Nanogels on Cotton Fabric	38
2.2.5 Characterizations	38
2.2.5.1 Scanning Electron Microscopy (SEM) and EDX Analysis	38
2.2.5.2 Antimicrobial Studies	39
2.2.5.3 Zone of Inhibition Analysis	39
2.2.5.4 Adherence Studies	39
2.2.5.5 Colony Count Method	40
2.2.5.6 Time Kill Assay	40

2.2.5.7 Live/ Dead Viability Assay	40
2.2.5.8 Transmission Electron Microscopy (TEM) with EDX Analysis	41
2.3 Results and Discussion	41
2.4 Conclusions	61
2.5 References	62
 Chapter 3: Preparation and Characterization of Sodium Alginate:Glycerol Blends	
3.1 Introduction	67
3.2 Experimental	68
3.2.1 Materials	68
3.2.2 Preparation of SA:Gly Blends	69
3.2.3 Rheology Studies	69
3.2.4 Fourier Transform Infrared (FTIR) Spectroscopy Analysis	69
3.2.5 Contact Angle Analysis	69
3.2.6 X-Ray Diffraction (XRD) Analysis	69
3.2.7 Scanning Electron Microscopy (SEM) Analysis	69
3.2.8 Mechanical Analysis	70
3.2.9 Coating of SA:Gly Blend on Cotton Fabric	70
3.2.10 Scanning Electron Microscopy (SEM) Analysis	70
3.2.11 Add on Analysis	70
3.3 Results and Discussion	71
3.4 Conclusions	80
3.5 References	81

Chapter 4: Preparation and Characterization of Herbal loaded Antimicrobial Hemostatic Dressings

4.1 Introduction	84
4.2 Experimental	87
4.2.1 Materials	87
4.2.2 Preparation of <i>Choerospondias axillaris</i> and <i>Mesua ferrea</i> Aqueous Extract	87
4.2.3 Fabrication of Herbal loaded Antimicrobial Hemostatic Dressings	88
4.2.4 Characterizations	88
4.2.4.1 Antioxidant Assay	88
4.2.4.2 <i>In-vitro</i> Blood Clotting Analysis	89
4.2.4.3 Blood Clotting Time Analysis	89
4.2.4.4 Blood Clotting Index (BCI) Analysis	90
4.2.4.5 PT and aPTT Test	90
4.2.4.6 Red Blood Cells (RBC) Adhesion Analysis	90
4.2.4.7 Antimicrobial Analysis	91
4.2.4.8 Zone of Inhibition Analysis	91
4.2.4.9 Adherence Studies	91
4.2.4.10 Colony Count Method	92
4.2.4.11 Transmission Electron Microscopy (TEM)	92
4.2.4.12 Cytotoxicity Studies	93
4.2.4.13 Scanning Electron Microscopy (SEM) Analysis	93
4.2.4.14 Energy Dispersive X-ray (EDX) Spectroscopy Analysis	94
4.2.4.15 Contact Angle Analysis	94
4.3 Results and Discussion	94
4.4 Conclusions	118

4.5 References	119
----------------	-----

Chapter 5: *In-vivo* Studies of Herbal loaded Antimicrobial Hemostatic Dressings

5.1 Introduction	124
5.2 Experimental	126
5.2.1 Materials and Animals	126
5.2.2 Blood Clotting Time Analysis	126
5.2.3 Blood Loss Studies	127
5.2.4 Statistical Analysis	127
5.3 Results and Discussion	127
5.4 Conclusions	130
5.5 References	131

Chapter 6: Summary and Future Prospects

6.1 Summary	133
6.2 Future Prospects	136

Curriculum Vitae	137
-------------------------	-----

LIST OF FIGURES

Chapter 1

Figure 1.1: Schematic representation of the hemostatic mechanism. (A) Normal blood vessel, (B) Injured blood vessel.

Figure 1.2: Structure of polysaccharides used for development of hemostatic materials.

Figure 1.3: Structure of synthetic polymers used for the development of hemostatic materials.

Figure 1.4: Schematic representation indicating the role of sodium alginate in hemostasis.

Figure 1.5: Structure of tannic acid.

Figure 1.6: Digital image of *Choerospondias axillaris* fruit.

Figure 1.7: Digital image of *Mesua ferrea* plant.

Chapter 2

Figure 2.1: Schematic representation for the preparation of CMC-Ag nanogel coated fabric.

Figure 2.2: EDX analysis of CMC-Ag nanogel.

Figure 2.3: The UV-vis spectroscopy analysis of CMC-Ag nanogel at different CMC concentrations.

Figure 2.4: TEM micrographs and particle size distribution curve of CMC-Ag nanogels with CMC concentration of (A) 0.05%, (B) 0.1%, (C) 0.5%. Time: 5 min; Temperature: 40°C.

Figure 2.5: UV-vis spectroscopy analysis of CMC-Ag nanogels at different reduction times.

Figure 2.6: TEM images and histograms for particle size distribution of CMC-Ag nanogel with the reduction time (A) 5 min, (B) 30 min, (C) 60 min. CMC concentration: 0.1%; Temperature: 40°C.

Figure 2.7: The UV-vis spectroscopy analysis of CMC-Ag nanogel at different reaction temperatures.

Figure 2.8: TEM images and histograms for particle size distribution of CMC-Ag nanogel with the reduction temperatures (A) 40°C, (B) 60°C, (C) 80°C. CMC concentration: 0.1%; Time: 30 min.

Figure 2.9: FESEM images of (a) Pure CMC and CMC-Ag nanogels at different reaction temperature, (b) 40°C, (c) 60°C, (d) 80°C.

Figure 2.10: Particle size analysis of CMC-Ag nanogels at different reduction temperatures by DLS measurements.

Figure 2.11: Zeta potential analysis of CMC-Ag nanogels at different reduction temperatures by DLS studies.

Figure 2.12: Digital images representing the change in color of CMC-Ag nanogels with different silver nitrate concentrations.

Figure 2.13: Digital and SEM images of (a) Pristine fabric, (b) CMC-Ag nanogel coated fabric.

Figure 2.14: EDX analysis of CMC-Ag nanogel immobilized fabric in mapping mode.

Figure 2.15: Pictograph of the zone of inhibition of coated fabrics with varying nanogel content. (a) CMC-Ag100, (b) CMC-Ag200, (c) CMC-Ag300, (d) CMC-Ag400.

Figure 2.16: Histogram for the zone of inhibition diameter (mm) of coated fabrics.

Figure 2.17: SEM micrographs showing bacterial adhesion on the fabric. (a) Control, (b) CMC-Ag100, (c) CMC-Ag200, (d) CMC-Ag300, (e) CMC-Ag400.

Figure 2.18: Colony count method analysis of *S. aureus* and *E. coli*. (a) Control, (b) CMC-Ag100, (c) CMC-Ag200, (d) CMC-Ag300, (e) CMC-Ag400.

Figure 2.19: Quantitative evaluation of colony count method. (a) *S. aureus*, (b) *E. coli*.

Figure 2.20: Investigation of live-dead bacterial cells of *S. aureus* and *E. coli* by confocal microscopy analysis at 40X magnification. (a) Control (b) CMC-Ag300 fabric.

Figure 2.21: Time kill curves after incubation with control and CMC-Ag300 fabric treated samples. (a) *S. aureus*, (b) *E. coli*.

Figure 2.22: Morphological analysis of bacterial cells. (a) Control, (b) CMC-Ag300 fabric.

Figure 2.23: EDX spectrum of bacterial cells. (a) Control, (b) CMC-Ag300 fabric.

Figure 2.24: Elemental mapping analysis showing the presence of silver in green colour. (a) *S. aureus*, (b) *E. coli*.

Figure 2.25: Digital images showing the viable colonies of bacteria after incubation with control and CMC-Ag300 fabric samples.

Figure 2.26: Histograms for the viable colony reduction percentage at different time points.

Figure 2.27: HR-TEM micrograph of CMC-Ag300 nanogel at the magnification of 240KX.

Chapter 3

Figure 3.1: Schematic presentation of the SA:Gly blend preparation.

Figure 3.2: Digital images indicating the SA:Gly blend films with glycerol concentrations. (a) 0%, (b) 10%, (c) 20%, (d) 30%, (e) 40%.

Figure 3.3: Rheological behavior of SA:Gly blends with different glycerol concentrations.

Figure 3.4: FTIR spectra of SA:Gly films with varying concentrations of glycerol.

Figure 3.5: Contact angle analysis of SA:Gly films with varying concentrations of glycerol.

Figure 3.6: XRD diffractograms of SA:Gly films with varying concentrations of glycerol.

Figure 3.7: SEM micrographs of SA:Gly films with varying glycerol concentrations. (a) 0%, (b) 10%, (c) 20%, (d) 30%, (e) 40%.

Figure 3.8: Stress vs. strain curves of SA:Gly films.

Figure 3.9: Tensile strength and modulus of films as a function of the glycerol concentrations.

Figure 3.10: SEM micrographs of cotton fabric coated with SA:Gly blends with increasing concentrations of glycerol. (a) 0%, (b) 10%, (c) 20%, (d) 30%, (e) 40%.

Figure 3.11: Histogram indicating the add on percentage of SA:Gly blends coated cotton fabric.

Chapter 4

Figure 4.1: Schematic diagram presenting the preparation process of antimicrobial hemostatic dressings.

Figure 4.2: Free radical scavenging activity of control as well as NGSG-TA dressings by DPPH assay.

Figure 4.3: Antioxidant activity of NGSG-CA dressings by DPPH assay.

Figure 4.4: Antioxidant activity of NGSG-MF dressings by DPPH assay.

Figure 4.5: Digital pictographs indicating the blood clotting time of dressings with varying concentrations of TA.

Figure 4.6: Digital images indicating the blood clotting by fabricated dressings with varying concentrations of CA as a function of time.

Figure 4.7: Digital micrographs of developed dressings with varying concentrations of MF indicating the blood clotting time.

Figure 4.8: Histograms indicating the blood clotting index (%) of NGSG-TA dressings.

Figure 4.9: Histograms corresponding to the blood clotting index (%) of NGSG-CA dressings.

Figure 4.10: Histograms showing the blood clotting index (%) of NGSG-MF dressings.

Figure 4.11: Histogram indicating the effect of NGSG-TA3, NGSG-CA3 and NDGS-MF3 on PT.

Figure 4.12: Histogram indicating the effect of NGSG-TA3, NGSG-CA3 and NDGS-MF3 on aPTT.

Figure 4.13: FESEM micrographs of RBC adhesion on the dressings surface.

Figure 4.14: Digital images indicating the zone of inhibition for both the bacterial strains *S. aureus* and *E. coli*.

Figure 4.15: Histogram for the Zone of Inhibition diameter (mm) of NG and NGSG-TA3 group against *S. aureus* and *E. coli*.

Figure 4.16: Digital images showing zone of inhibition against *S. aureus* and *E. coli*.

Figure 4.17: Histogram for the zone of inhibition diameter (mm) of NG and NGSG-CA3 group for *S. aureus* and *E. coli*.

Figure 4.18: Digital pictographs representing the zone of inhibition against *S. aureus* and *E. coli*.

Figure 4.19: Histogram for the zone of inhibition diameter (mm) of NG and NGSG-MF3 groups.

Figure 4.20: SEM micrographs representing the adhesion of *S. aureus* on the control as well as antimicrobial hemostatic dressing surface.

Figure 4.21: SEM micrographs representing the adhesion of *E. coli* on the control as well as antimicrobial hemostatic dressing surface.

Figure 4.22: Digital images indicating the antimicrobial nature of dressings.

Figure 4.23: Digital images indicating the antimicrobial nature of dressings.

Figure 4.24: Histograms indicating the quantitative evaluation of antimicrobial activity of NGSG-TA3 dressings against *S. aureus* and *E. coli*.

Figure 4.25: Histograms indicating the quantitative evaluation of antimicrobial activity of NGSG-CA3 dressings against *S. aureus* and *E. coli*.

Figure 4.26: Histograms indicating the quantitative evaluation of antimicrobial activity of NGSG-MF3 dressings against *S. aureus* and *E. coli*.

Figure 4.27: TEM micrographs indicating the bacterial cell morphology against *S. aureus*.

Figure 4.28: TEM micrographs indicating the bacterial cell morphology against *E. coli*.

Figure 4.29: Elemental mapping analysis showing the presence of silver in *S. aureus* bacterial strain treated with dressings.

Figure 4.30: Electron microscopic images with elemental mapping mode showing the presence of silver in *E. coli* bacterial strain treated with dressings.

Figure 4.31: Histograms indicating the cell viability (%) determined by the MTT assay.

Figure 4.32: Morphology of NIH3T3 cells cultured with dressings for 24 h. (a) bright field, (b) confocal microscopy images.

Figure 4.33: SEM micrograph indicating the surface morphology. (a) Pristine, (b) NGSG-TA3, (c) NGSG-CA3, (d) NGSG-MF3.

Figure 4.34: EDX analysis of hemostatic dressings in mapping mode. (A) NGSG-TA3, (B) NGSG-CA3, (C) NGSG-MF3.

Figure 4.35: Contact angle analysis indicating the highly hydrophilic nature of the dressings.

Chapter 5

Figure 5.1: Digital images indicating the establishment of tail amputation model. (a) Measurement of tail length, (b) Cut on the half-length, (c) Bleeding model.

Figure 5.2: Digital micrographs of tail amputation model. (a) Control, (b) NGSG, (c) Axiostat[®], (d) NGSG-MF3 (e) NGSG-TA3, (f) NGSG-CA3.

Figure 5.3: Histograms indicating the hemostatic time (s) analysis.

Figure 5.4: Histograms indicating the amount of blood loss (g).

LIST OF TABLES

Chapter 1

Table 1.1: Commercially available FDA approved hemostats.

Chapter 3

Table 3.1: Influence of the glycerol addition on the tensile properties of SA:Gly films.

Chapter 5

Table 5.1: Details of groups divided for the tail amputation test.

LIST OF ABBREVIATIONS

Expanded Form	Abbreviation
Fourier transform-infrared spectroscopy	FTIR
X-ray diffraction	XRD
Scanning electron microscopy	SEM
Transmission electron microscopy	TEM
Field emission scanning electron microscopy	FESEM
Energy-Dispersive X-ray Spectroscopy	EDX
Dynamic light scattering	DLS
High resolution transmission electron microscopy	HRTEM
Zone of inhibition	ZOI
Colony forming unit	CFU
Escherichia coli	E. coli
Staphylococcus aureus	S. aureus
Carboxymethyl cellulose	CMC
Silver nanoparticles	AgNP
CMC-Ag300 nanogel immobilized cotton fabric	NG
Sodium alginate	SA
Glycerol	Gly
SA:Gly (70:30) blend	SG
Blood clotting index	BCI
Red blood cells	RBC
Choerospondias axillaris	CA
Mesua ferrea	MF

Tannic acid	TA
Prothrombin time	PT
Tissue Factors	TF
Activated partial thromboplastin time	aPTT
Phosphate buffer saline	PBS
Polyvinyl alcohol	PVA
Polyethylene glycol	PEG
Polyvinylpyrrolidone	PVP
poly(-L-glutamic acid)	PGA
Propidium iodide	PI
Acridine orange	AO
Diphenyl picrylhydrazyl	DPPH
Dulbecco's modified Eagle's medium	DMEM
Fetal bovine serum	FBS
(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)	MTT
Sodium-montmorillonite	MMT